

University of Wollongong

Research Online

Faculty of Science, Medicine and Health -
Papers: part A

Faculty of Science, Medicine and Health

1-1-2015

Radical formation in the gas-phase ozonolysis of deprotonated cysteine

George N. Khairallah
University of Melbourne

Alan T. Maccarone
University of Wollongong, alanmac@uow.edu.au


Huong T. Pham
University of Wollongong, thp658@uowmail.edu.au

Timothy M. Benton
University of Melbourne

Tony Ly
University of Wollongong, tly@uow.edu.au

See next page for additional authors

Follow this and additional works at: <https://ro.uow.edu.au/smhpapers>

 Part of the [Medicine and Health Sciences Commons](#), and the [Social and Behavioral Sciences Commons](#)

Recommended Citation

Khairallah, George N.; Maccarone, Alan T.; Pham, Huong T.; Benton, Timothy M.; Ly, Tony; da Silva, Gabriel; Blanksby, Stephen J.; and O'Hair, Richard A. J, "Radical formation in the gas-phase ozonolysis of deprotonated cysteine" (2015). *Faculty of Science, Medicine and Health - Papers: part A*. 3397.
<https://ro.uow.edu.au/smhpapers/3397>

Research Online is the open access institutional repository for the University of Wollongong. For further information contact the UOW Library: research-pubs@uow.edu.au

Radical formation in the gas-phase ozonolysis of deprotonated cysteine

Abstract

Although the deleterious effects of ozone on the human respiratory system are well-known, many of the precise chemical mechanisms that both cause damage and afford protection in the pulmonary epithelial lining fluid are poorly understood. As a key first step to elucidating the intrinsic reactivity of ozone with proteins, its reactions with deprotonated cysteine [Cys-H]⁻ are examined in the gas phase. Reaction proceeds at near the collision limit to give a rich set of products including 1) sequential oxygen atom abstraction reactions to yield cysteine sulfenate, sulfinic and sulfonate anions, and significantly 2) sulfenate radical anions formed by ejection of a hydroperoxy radical. The free-radical pathway occurs only when both thiol and carboxylate moieties are available, implicating electron-transfer as a key step in this reaction. This novel and facile reaction is also observed in small cys-containing peptides indicating a possible role for this chemistry in protein ozonolysis.

Disciplines

Medicine and Health Sciences | Social and Behavioral Sciences

Publication Details

Khairallah, G. N., Maccarone, A. T., Pham, H. T., Benton, T. M., Ly, T., da Silva, G., Blanksby, S. J. & O'Hair, R. A. J. (2015). Radical formation in the gas-phase ozonolysis of deprotonated cysteine. *Angewandte Chemie International Edition*, 54 (44), 12947-12951.

Authors

George N. Khairallah, Alan T. Maccarone, Huong T. Pham, Timothy M. Benton, Tony Ly, Gabriel da Silva, Stephen J. Blanksby, and Richard A. J O'Hair

Radical Formation in the Gas-Phase Ozonolysis of Deprotonated Cysteine.

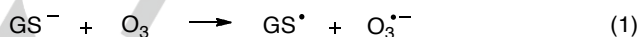
George N. Khairallah,^{[a]‡} Alan T. Maccarone,^{[b]‡} Huong T. Pham,^[b] Timothy M. Benton,^[a] Tony Ly,^[b] Gabriel da Silva,^[c] Stephen J. Blanksby,^{[d]*} and Richard A. J. O'Hair,^{[a]*}

Abstract: Although the deleterious effects of ozone on the human respiratory system are well known, many of the precise chemical mechanisms that both cause damage and afford protection in the pulmonary epithelial lining fluid are poorly understood. As a key first step to elucidating the intrinsic reactivity of ozone with proteins, its reactions with deprotonated cysteine [Cys-H][−] are examined in the gas phase. Reaction proceeds at near the collision limit to give a rich set of products including (i) sequential O atom abstraction reactions to yield cysteine sulfenate, sulfinate and sulfonate anions, and significantly (ii) sulfenate radical anions formed by ejection of a hydroperoxyl radical. The free radical pathway occurs only where both thiol and carboxylate moieties are available implicating electron-transfer as a key step in this reaction. This novel and facile reaction is also observed in small cys-containing peptides indicating a possible role for this chemistry in protein ozonolysis.

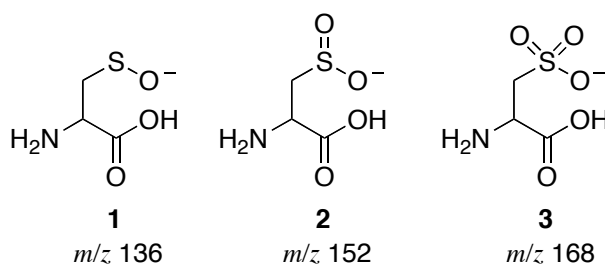
Ozone (O₃), a triatomic allotrope of oxygen, plays two opposing roles for human life depending on its location in the Earth's atmosphere. In the upper atmosphere (stratosphere) O₃ is beneficial in preventing UV light from reaching the surface of the Earth, thereby minimising human exposure to damaging wavelengths in the solar spectrum.¹ In contrast, in the lower atmosphere (troposphere) O₃ is a product of photochemical smog and its role changes to that of an air pollutant with deleterious effects to the human respiratory system.¹ Long-term exposure to elevated O₃ levels has been linked to increased risk of death from respiratory causes, which is more than three times greater in metropolitan areas with high tropospheric O₃ concentrations (62.5–104.0 ppb) compared to those with lower levels (33.3–53.1 ppb).² This association is concerning given predictions that background O₃ levels may exceed internationally accepted levels for human health this century.³

While the physiological and biochemical outcomes of O₃ inhalation are well studied, the underlying chemical mechanisms are less well characterised.⁴ This is in large part because the interaction of O₃ with the lung surfactant layer is thought to give rise to a cascade of secondary reactive species, which may ultimately be responsible for the bulk of the damaging effects.⁵

Significant effort has been expended to characterise the interaction of O₃ with unsaturated lipids present in pulmonary surfactant and indeed some of these processes have been shown to release detectable free radicals *in vitro*.⁵ The nature of the interaction of O₃ with the peptides and proteins – also key components of lung surfactants – is less well understood. It has been hypothesised that thiols present in biomolecules such as pulmonary surfactant B and glutathione (GSH) could serve as sacrificial antioxidants giving rise to the largely benign sulfonates. Conversely, reaction of O₃ with glutathione has been shown to mediate the release of damaging hydroxyl radicals. Although the precise mechanism of this process has not been fully elucidated, a key first step is believed to involve electron transfer (*e.g.*, eq. 1, where GS[−] is deprotonated glutathione).⁵



To better understand the effects of O₃ on the respiratory tract, recent studies have examined the ozonolysis of amino acids, peptides and proteins.^{6–8} While most studies monitor the end products of solution-phase reactions, novel mass spectrometric approaches have probed the interaction of O₃ with biomolecules at an air-liquid interface.⁸ These phase-boundary experiments confirm that cysteine residues are preferential sites of oxidation in peptides and proteins under conditions relevant to the interaction of ambient O₃ with lung surfactant. Thermospray ionization tandem mass spectrometry identified the following key intermediates produced in the interfacial ozonolysis of deprotonated cysteine: cysteine sulfenate, (*m/z* 136, **1** of Scheme 1); cysteine sulfinate, (*m/z* 152, **2**); and cysteine sulfonate, (*m/z* 168, **3**). In addition, product ions at *m/z* 135 and *m/z* 271 were tentatively assigned as being due to [M-2H]^{2−} and [M-H][−] of cystine oxide, respectively.^{8d}



Scheme 1. Structures of oxidation products formed via ozonolysis of deprotonated cysteine as identified by Colussi and co-workers.^{8d}

These observations raise intriguing questions as to the: mechanism of the reaction(s) involved; precise nature of the oxidant in each case (*i.e.*, O₃ or secondary oxidants); identity of all the reaction intermediates and products. To address these questions, we have studied the gas-phase ion-molecule reaction of deprotonated cysteine (and its derivatives) with O₃ using a combination of multistage mass spectrometry experiments,

[a] Dr. G. N. Khairallah, Mr. T. M. Benton, Prof. R. A. J. O'Hair
School of Chemistry and Bio21 Institute, University of Melbourne
Melbourne, Victoria 3010 (Australia) and ARC Centre of Excellence
for Free Radical Chemistry and Biotechnology
E-mail: rohair@unimelb.edu.au

[b] Dr. A. T. Maccarone, Dr. H. T. Pham, Dr. T. Ly
School of Chemistry, University of Wollongong, New South Wales
2522, Australia.

[c] Dr. G. da Silva
Department of Chemical and Biomolecular Engineering, The
University of Melbourne, Melbourne, Victoria 3010, Australia.

[d] Prof. S. J. Blanksby
Central Analytical Research Facility, Queensland University of
Technology, Queensland 4001 (Australia) and ARC Centre of
Excellence for Free Radical Chemistry and Biotechnology

Supporting information for this article is given via a link at the end of
the document.

‡These authors contributed equally.

deuterium and structural labeling and high-resolution mass spectrometry (HRMS).⁹

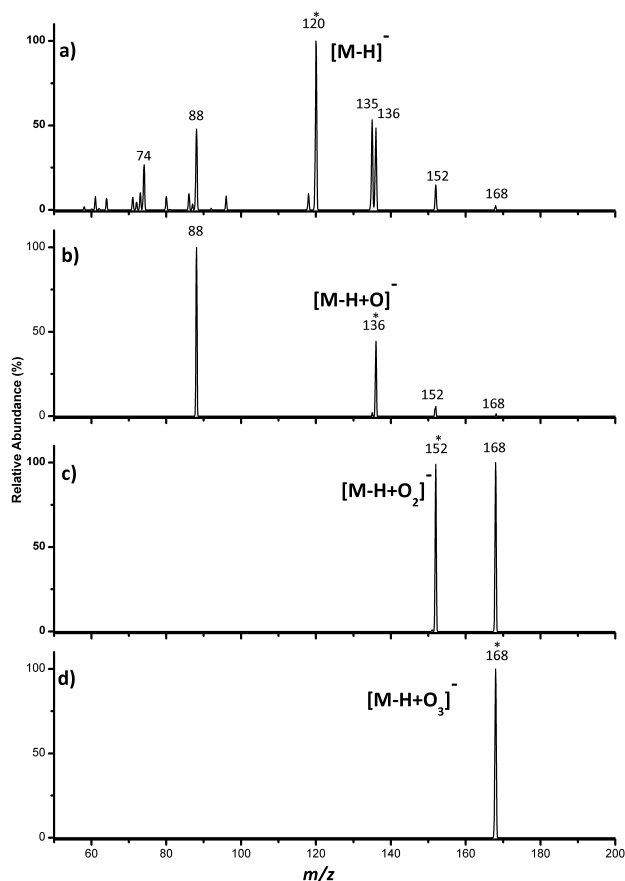
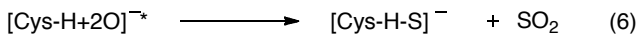
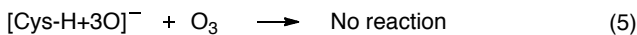
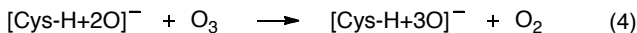
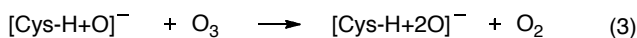
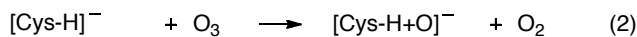


Figure 1. Mass spectra obtained following isolation of the following cysteine derived anions (marked with *) in the presence of ozone: (a) $[\text{Cys-H}]^-$ at m/z 120; (b) $[\text{Cys-H+O}]^-$ at m/z 136; (c) $[\text{Cys-H+2O}]^-$ at m/z 152; and (d) $[\text{Cys-H+3O}]^-$ at m/z 168.

Using linear ion-trap mass spectrometers recently modified to examine ion-molecule reactions,⁹ deprotonated cysteine ($[\text{Cys-H}]^-$ at m/z 120) was transferred to the gas phase via electrospray ionization, mass-selected and allowed to react with O_3 within the ion trap, resulting in a product-rich spectrum (Figure 1a). Product ions observed include the previously reported oxides $[\text{Cys-H+O}]^-$ (**1** m/z 136), $[\text{Cys-H+2O}]^-$ (**2** m/z 152), $[\text{Cys-H+3O}]^-$ (**3** m/z 168) together with a range of other species at m/z 135 (**4**), 88 (**5**) and 74 (**6**). Sequential isolation of the oxides and subsequent reactions with O_3 (Figures 1b-d) reveal that: (i) the

higher oxides can be formed *via* sequential oxygen atom transfer reactions (eqs 2 - 4); (ii) ozonolysis terminates with $[\text{Cys-H+3O}]^-$, which does not react further with O_3 (Figure 1d and eq. 5); (iii) the ion observed at m/z 88 in Figure 1(a) arises at least in part *via* loss of SO_2 from nascent $[\text{Cys-H+2O}]^-$ (eq. 6); (iv) the remaining product ions at m/z 74 and 135 are either primary product ions in the ozonolysis of deprotonated cysteine, or fragments formed from activated $[\text{Cys-H+O}]^-$ (*vide infra*).

HRMS allowed assignment of the empirical formulas of m/z 74 (**6**) and 88 (**5**) as being the even-electron species $\text{C}_2\text{H}_4\text{NS}$ and $\text{C}_3\text{H}_6\text{NO}_2$, while m/z 135 (**4**) was confirmed to be a radical anion, $\text{C}_3\text{H}_5\text{NO}_3\text{S}$ (see Supporting Information Table S1). A recent report on the gas phase reaction of deprotonated cysteine and singlet molecular oxygen ($^1\Delta_g$) assigned a m/z 135 product ion to the sulfinyl radical anion $\text{NH}_2\text{CH}(\text{CH}_2\text{SO}^\bullet)\text{CO}_2^-$ which is consistent with the high-resolution data here, but attributed a m/z 74 product ion to the glycyl anion $\text{NH}_2\text{CH}_2\text{CO}_2^-$ suggesting it has been miss-assigned or there is significant divergence in chemistries of the two oxygen allotropes with the cysteine anion.¹⁰ The possible origins of the products **4-6** arising from ozonolysis were investigated by isolating each of the oxides $[\text{Cys-H+nO}]^-$ ($n = 1 - 3$) and subjecting them to activation by collision-induced dissociation (CID) (see Supporting Information Figure S1). $[\text{Cys-H+O}]^-$ dissociates to give both m/z 74 and 88, whereas m/z 88 is the sole fragment of $[\text{M-H+2O}]^-$. The CID spectrum of $[\text{Cys-H+3O}]^-$ is dominated by loss of ammonia, with no formation of products at m/z 74 and 88. These tandem mass spectra are consistent with those reported by Colussi and co-workers for cysteine oxides formed upon ozonolysis at the air-liquid interface^{8d} and confirm the identity of these ions as cysteine sulfenate (**1**); sulfinate (**2**); and sulfonate (**3**) (Scheme 1). These experiments further suggest that the even-electron ions at m/z 74 and 88 observed in Figure 1(a) arise from spontaneous dissociation of vibrationally hot $[\text{Cys-H+O}]^-$ and $[\text{Cys-H+2O}]^-$. In contrast, the $[\text{Cys-2H+O}]^{*-}$ radical anion at m/z 135 cannot be formed by dissociation of any of the cysteine oxides, $[\text{Cys-H+nO}]^-$ ($n = 1 - 3$), suggesting that it is a primary product formed by addition of O_3 with ejection of the hydroperoxyl radical (eq. 7).

To further explore the mechanism associated with the formation of the radical anion **4**, deuterium and structural labelling was carried out. Condensed phase hydrogen-deuterium exchange of all the four labile, heteroatom protons of cysteine (*i.e.*, at $-\text{NH}_2$, $-\text{CO}_2\text{H}$ and $-\text{SH}$) followed by electrospray ionization produced the labeled $[\text{D}_4\text{-Cys-D}]^-$ anion at m/z 123. This isotopologue was found to react with O_3 to yield, amongst other products, $[\text{D}_4\text{-Cys-2D+O}]^-$ at m/z 137 and $[\text{D}_4\text{-Cys-D+O}]^-$ at m/z 139 (see Supporting Information Table S2) thus confirming the exclusive loss of one of the four exchangeable protons in the formation of **4**. Since previous gas-phase studies have suggested that the two most acidic sites in cysteine are the $-\text{CO}_2\text{H}$ and $-\text{SH}$ moieties,¹¹ derivatives in which each of these sites are blocked by methylation were investigated. Ozonolysis of deprotonated *S*-methyl cysteine (m/z 134) produces $[\text{Cys}(\text{SCH}_3)\text{-H+O}]^-$ (m/z 150) as the major product and no odd-electron product ions are observed (see Supporting Information Figure S2a). The reaction is poorly efficient ($\Phi = 3\%$, Table 1) compared to the cysteine archetype ($\Phi = 89\%$). Deprotonated

cysteine *O*-methyl ester shows an initial rapid reaction with O₃ that leads to a 40% loss of ion signal. Following this initial phase a persistent, non-reactive *m/z* 134 ion population is observed (see Supporting Information, Figure S3). High-resolution measurements (data not shown) exclude the presence of isobars, suggesting that two isomers are present within the ion population or that O₃ catalyses the isomerisation of the nascent [Cys(OCH₃)-H][−] anions. Significantly, the absence of abundant odd-electron product ions in the reactions of both [Cys(SCH₃)-H][−] and [Cys(OCH₃)-H][−] implicates both the carboxylic acid and thiol functional groups in the free radical pathways observed for [Cys-H][−]. In contrast, blocking of the amine moiety in *N*-acetyl cysteine, was found to yield odd and even-electron products analogous to those observed for cysteine (see Supporting Information Figure S2b). The effect of structural variation on reaction efficiency was also measured. In general, anions with a free thiol were found to react efficiently with O₃ (e.g., [Cys-H][−], and [Cys(OCH₃)-H][−] see Table 1) with the efficiency dropping markedly for sulfides (e.g., [Cys(SCH₃)-H][−] and [Met-H][−]) and slowly reducing with increasing oxidation of the sulfur (e.g., [Cys-H+O][−], [Cys-H+2O][−]).

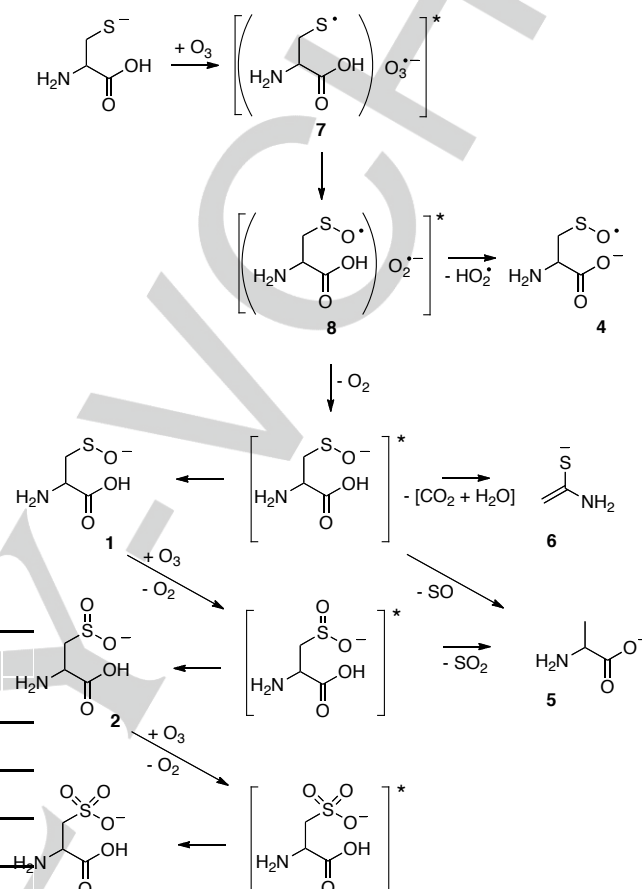
Table 1. Second order rate constants and efficiencies measured for the reaction of deprotonated cysteine analogues with ozone in the gas phase. Rate constants were determined by measuring the decay of the precursor ion in the presence of ozone using increasing reaction times in a modified linear ion-trap mass spectrometer. The concentration of ozone inside the ion trap was determined using reference reactions (Supporting Information).

Reactant anion ^[a]	<i>m/z</i>	Rate constant (<i>k</i> ^{II}), ×10 ^{−11} cm ³ molecules ^{−1} s ^{−1} ^[a]	Reaction efficiency (Φ), % ^[b]
[Cys-H] [−]	120	77 (± 19)	89
[Cys-H+O] [−]	136	50 (± 12)	59
[Cys-H+2O] [−]	152	18 (± 4)	22
[Cys(SCH ₃)-H] [−]	134	3 (± 1)	3
[Cys(OMe)-H] [−]	134	>77 (± 19) ^c	>89
[Cys(NAc)-H] [−]	162	17 (± 4)	21
[Met-H] [−]	148	3 (± 1)	3
[CysGly-H] [−]	177	35 (± 9)	43
[CysGlyGly-H] [−]	234	30 (± 7)	38

[a] Uncertainties in the second order rate constants represent a least squares analysis of the standard error in the fit of the pseudo-first-order decay and the quoted uncertainty of ± 25% in the reference reaction.¹⁴ [b] Reaction efficiencies based on theoretical collision rates (Supporting Information). [c] See discussion in text and Supporting Information Figure S3.

The presence of cysteine in a deprotonated di- or tri-peptide facilitates efficient reaction with O₃; even as the carboxylate and thiol moieties are separated along the peptide chain (e.g., CysGly and CysGlyGly in Table 1). Ozonolysis of cysteine-containing peptides also yields abundant odd-electron [M-2H+O][−] ions (see Supporting Information Table S3). Similar sulfinyl radicals have been reported from radical-driven cleavage of disulfide linked-peptides in the gas phase¹² and have also been detected as reactive intermediates in some biochemical transformations.¹³

These labeling experiments together with known thermochemistry can be used to infer a mechanism for free radical formation in the ozonolysis of [Cys-H][−] (Scheme 2).



Scheme 2. Proposed mechanism for the formation of both [Cys-H+O][−] and [Cys-2H+O][−] from the reaction of ozone with [Cys-H][−]. Detailed mechanisms for formation of **6** and **5** are provided as Supporting Information Scheme S1. The asterisk (*) indicates energetic or activated intermediates.

While there has been much discussion related to the gas-phase structure of [Cys-H][−], stationary points for deprotonation at both -CO₂H and -SH have been identified and lie within a few kcal mol^{−1} of each other.^{10,15} The flat potential energy surface allows wide sampling of anion structures within the ion-dipole complex. Accessible conformers with a formal charge on sulfur will have lower electron binding energies than isomeric carboxylate anions (i.e., 2.4 eV for [Cys(OCH₃)-H][−] versus 3.4 eV for [Cys(SCH₃)-H][−]).^{11a} The comparative stability of sulfinyl radicals means that the intra-complex electron transfer (ET) from cysteine to ozone (*EA*[O₃] = 2.1 eV)¹⁶ is only slightly endothermic and can readily be fueled by the initial complexation energy. The participation of the S anion is confirmed by structural labeling showing that the free radical products are not observed for deprotonated *S*-methyl cysteine (see Figure S2a). Following ET, oxygen atom abstraction yields the modified ion-molecule complex **8**, which can dissociate via

either (i) electron transfer to give molecular oxygen and [Cys-H+O][•] or (ii) proton transfer to give the hydroperoxyl radical and **4**. In the absence of an acidic hydrogen atom in [Cys(OCH₃)-H][•], the latter pathway is shut down and only even-electron products observed. Scheme 2 also outlines the subsequent oxidation of the cysteine sulfenate anion (**1**) to yield the higher sulfinate (**2**) and sulfonate (**3**) homologues but the absence of the free sulfur inactivates the radical forming pathway. The importance of initial ET in this mechanism is supported by the absence of reactivity of the protonated form of cysteine, [Cys+H]⁺ under the same reaction conditions (Supporting Information, Figure S4). The slow oxygen atom abstraction reactions of [Cys(SCH₃)-H][•] and [Met-H][•] are likely to rise from other mechanistic pathways.

Modeling of the reaction kinetics provides a branching ratio (BR) of 4% for the free radical pathway resulting in **4** (see Supporting Information, Figure S5 and Table S4). This compares to BRs of 10% and 36% for even-electron pathways resulting in product ions **1** and **6**, respectively. Thus almost half of all reactive encounters between [Cys-H][•] and O₃ result in products undetectable by ion-trap MS, which may arise from efficient reaction pathways resulting in electron loss with concomitant formation of neutral free radical(s).

The observations of the gas-phase reactions between [Cys-H][•] and O₃ demonstrates that (i) even-electron oxidation proceeds *via* facile, sequential oxygen atom abstraction reactions and (ii) free radical products are also formed via the addition of oxygen and the elimination of HO₂[•]. The products arising from the intrinsic gas-phase reactivity of cysteine toward O₃ show excellent agreement with prior observations of the interfacial ozonolysis suggesting the identity of the *m/z* 135 reaction product is the radical ion **4** rather than an even-electron dianion of cystine as previously proposed.^{8d} Given the gas-phase ozonolysis of cysteine containing peptides also yields free radicals, such pathways may also operate for the interfacial reactions of cysteine containing proteins. The efficiency (*i.e.*, 89%, Table 1) of the gas-phase reaction between [Cys-H][•] and O₃ is approximately two orders of magnitude greater than for the ozonolysis of ionised unsaturated lipids under the same conditions.¹⁷ This is consistent with observations of facile and selective oxidation of pulmonary surfactant protein B at the air-liquid interface even in the presence of an excess of unsaturated phospholipids,^{8a} although further work is required to confirm free radical formation at the air-liquid interface of the lung.

The unequivocal identification of free radical products in the intrinsic reaction of deprotonated cysteine (and its derivatives) with O₃ is consistent with the proposal of secondary reactive species being responsible for promulgating cellular damage in the lung upon O₃ exposure: the so-called "cascade mechanism" for O₃ toxicity.¹⁸ Analogous chemistry occurring within the epithelial lining would release hydroperoxyl radicals initiating lipid peroxidation¹⁹ and cysteine sulfinyl radicals that have been implicated in enzyme inactivation pathways.²⁰

Experimental Section

Experiments were carried out using a modified Thermo Fisher Scientific LTQ single stage linear ion-trap mass spectrometer (San Jose, CA, USA),^{9a} and a modified Thermo LTQ-FT (Bremen, Germany).^{9b} Full details of the experiments are described in the Supporting Information.

Acknowledgements

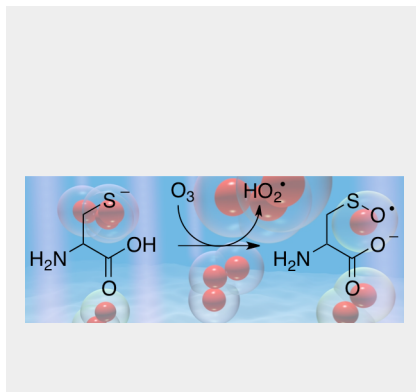
The authors are grateful to the Australian Research Council for funding through the Discovery Project (DP140101237 [SJB]) and the Centre of Excellence in Free Radical Chemistry and Biotechnology (CE0561607). GNK is an ARC Australian Research Fellow (DP1096134) and GdS is an ARC Future Fellow (FT130101304).

Keywords: ozone • cysteine • radicals • gas-phase reactions

- [1] B. J. Finlayson-Pitts and J. N. Pitts, Jr., *Chemistry of the Upper and Lower Atmosphere*, Academic Press, San Diego, **2000**.
- [2] M. Jerrett, R. T. Burnett, C. A. Pope, K. Ito, G. Thurston, D. Krewski, Y. L. Shi, E. Calle, M. Thun, *N. Engl. J. Med.* **2009**, *360*, 1085.
- [3] a) A. M. Hough, R. G. Derwent, *Nature* **1990**, *344*, 6267. b) R. Vingarzan, *Atmos. Environ.* **2004**, *38*, 3431.
- [4] K. M. Wynalda, R. C. Murphy, *Chem. Res. Toxicol.* **2010**, *23*, 108.
- [5] W. A. Pryor, *Free Radical Biol. Med.* **1994**, *17*, 451.
- [6] V. K. Sharma, N. J. D. Graham, *Ozone-Sci. Eng.*, **2010**, *32*, 81.
- [7] a) T. Kotiaho, M. N. Eberlin, P. Vainiotalo, R. Kostianien, *J. Am. Soc. Mass Spectrom.*, **2000**, *11*, 526. b) J. M. Spraggins, J. A. Lloyd, M. V. Johnston, J. Laskin, D. P. Ridge, *J. Am. Soc. Mass Spectrom.*, **2009**, *20*, 1579.
- [8] a) H. I. Kim, H. Kim, Y. S. Shin, L. W. Beegle, S. S. Jang, E. L. Neidholdt, W. A. Goddard, J. R. Heath, I. Kanik, J. L. Beauchamp, *J. Am. Chem. Soc.*, **2010**, *132*, 2254. b) H. I. Kim, H. Kim, Y. S. Shin, L. W. Beegle, S. S. Jang, E. L. Neidholdt, W. A. Goddard, J. R. Heath, I. Kanik, J. L. Beauchamp, *J. Phys. Chem. B*, **2010**, *114*, 9496. c) S. Enami, M. R. Hoffman, A. J. Colussi, *Chem. Res. Toxicol.*, **2009**, *22*, 35. d) S. Enami, M. R. Hoffman, A. J. Colussi, *J. Phys. Chem. B*, **2009**, *113*, 9356.
- [9] a) M. C. Thomas, T. W. Mitchell, D. G. Harman, J. M. Deeley, J. R. Nealon, S. J. Blanksby, *Anal. Chem.* **2008**, *80*, 303. b) A. K. Y. Lam, C. Li, G. N. Khairallah, B. B. Kirk, S. J. Blanksby, A. J. Trevitt, U. Wille, R. A. J. O'Hair, G. da Silva, *Phys. Chem. Chem. Phys.*, **2012**, *14*, 2417.
- [10] Y. Fang, F. Liu, R. Emre, J. Liu, *J. Phys. Chem. B*, **2013**, *117*, 2878.
- [11] a) H. K. Woo, K. C. Lau, X. B. Wang, L. S. Wang, *J. Phys. Chem. A*, **2006**, *110*, 12603. b) Z. Tian, A. Pawlow, J. C. Poutsma, S. R. Kass, *J. Am. Chem. Soc.*, **2007**, *129*, 5403. c) J. Oomens, J. D. Steill, B. J. Redlich, *J. Am. Chem. Soc.*, **2009**, *131*, 4310. d) A. F. DeBlase, S. R. Kass, M. A. Johnson, *Phys. Chem. Chem. Phys.*, **2014**, *16*, 4569.
- [12] L. Tan, H. Hu, J. S. Francisco, Y. Xia, *Angew. Chem. Int. Ed.* **2014**, *53*, 1887.
- [13] S. G. Reddy, K. K. Wong, C. V. Parast, J. Peisach, R. S. Magliozzo, J. W. Kozarich, *Biochem.* **1998**, *37*, 558.
- [14] S. Williams, M. F. Campos, A. J. Midey, S. T. Arnold, R. A. Morris, A. A. Viggiano, *J. Phys. Chem. A* **2002**, *106*, 997.
- [15] M. L. Stover, V. E. Jackson, M. H. Matus, M. A. Adams, C. J. Cassady, D. A. Dixon, *J. Phys. Chem. B* **2012**, *116*, 2905.
- [16] D. W. Arnold, C. S. Xu, E. H. Kim, D. M. Neumark, *J. Chem. Phys.* **1994**, *101*, 912.
- [17] H. T. Pham, A. T. Maccarone, J. L. Campbell, T. W. Mitchell, S. J. Blanksby, *J. Am. Soc. Mass Spectrom.* **2013**, *24*, 286.
- [18] C. A. Ballinger, R. Cueto, G. Squadrito, J. F. Coffin, L. W. Velsor, W. A. Pryor, E. M. Postlethwait, *Free Rad. Biol. Med.* **2005**, *38*, 515.
- [19] H. Yin, L. Xu, N. A. Porter, *Chem. Rev.* **2011**, *111*, 5944.
- [20] M. G. Bonini, O. Augusto, *J. Biol. Chem.* **2001**, *276*, 9749.

COMMUNICATION

Deprotonated cysteine reacts with ozone at near the collision limit to give a rich set of products including (i) sequential oxygen atom abstraction reactions to yield cysteine sulfenate, sulfinic and sulfonic anions, and significantly (ii) sulfenyl radical anions formed by ejection of a hydroperoxyl radical. The free radical pathway occurs only where both thiol and carboxylate moieties are available implicating electron-transfer as a key step in this reaction.



George N. Khairallah, Alan T. Maccarone, Huong T. Pham, Timothy M. Benton, Tony Ly, Gabriel da Silva, Stephen J. Blanksby,* and Richard A. J. O'Hair*

Page No. – Page No.

Radical Formation in the Gas-Phase Ozonolysis of Deprotonated Cysteine.